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BIOLOGICAL BULLETIN

NUCLEAR CHANGES IN THE REGENERATING SPINAL CORD OF THE TADPOLE OF RANA CLAMITANS.[1]

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CONTENTS.

I. Statement of the Problem.

The present paper gives the results of an histological study of the early stages of regeneration in the spinal cord of the frog tadpole, *Rana clamitans.* It deals especially with the degenerative nuclear changes immediately following the operation, and the phenomena of nuclear division in the formation of the new organ.

Fraisse (1885) studied these stages in several vertebrates in order to discover the origin of the regenerated tissues, and presented the following conclusions which may be used as a basis for a further detailed study.

"1. Sowohl bei Amphibien wie bei Reptilien sind verletzte Gewebe nur im Stande, wiederum gleichartig Gewebe zu erzeugen. Die Leukocyten übernehmen bei der Gewebsbildung nur die

[1] Contribution from the Zoological Laboratory of the University of Illinois, No. 37.

Function der Ernährung; ausserdem nehmen sie zerfallende Gewebsproducte auf und assimiliren dieselben, um sie an anderen Orten wieder zu deponiren. Niemals werden sie selbst zu fixen Gewebszellen, weder in der Bindesubstanz noch sonst wo.

"2. Sämmtliche der in Frage kommenden Gewebe der Amphibien und Reptilien sind im Stande, sich zu regeneriren; entweder direct aus ihren Elementen, oder aus einer Matrix, so lange diese Matrix unverletzt ist. Als Matrix für die Epidermis ist das Rete Malpighii, für das centrale Nervensystem das Epithel des Centralcanales, für die Muskulatur die Muskelkörperchen zu betrachten.

"3. Zuerst regeneriren sich Epithel und Bindegewebe; beides scharf getrennt, ursprünglich aus gleichartigen Zellen bestehend, die sich später differenziren."

There remains the further problem of the stages in the process by which the old organs at the cut surface replace their lost parts. Two distinct kinds of changes take place in this process, (1) degenerative and (2) regenerative. First the injured cells at the cut edge degenerate. Then follows regeneration proper, or the formation of the new organ from the remaining elements of the old.

There are three ways in which regeneration proper might take place. (1) The cells at the cut edge of each organ by dividing might extend outward, and in time form the completed organ: (2) the cells in front of the cut edge might wander backward; and (3) the cells in front of the cut edge might divide in situ and push backward the more distal cells. These possible methods of regeneration will be made clearer by a diagram of that part of the hollow neural tube extending forward from the cut (Fig. 1). If (1) (division of cells at the cut edge) were the method of regeneration, we should find after the operation that

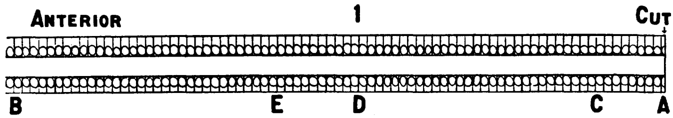

FIG. 1. Diagram, explained in the text.

the cells at the cut surface *A*, or from *A* to *C*, are dividing rapidly while from *C* to *B* about the normal number of cells is dividing.

If (2) (migration of cells) were the method, we might find no dividing cells at all, but should expect to find that the cells from *B* to *A* or possibly only from *D* to *A* are turned with their long axes parallel to the longitudinal axis of the spinal cord as if moving toward the cut end. If (3) (division of more anterior cells in situ) were the method, we should expect to find dividing cells all the way from *B* to *C* or possibly concentrated in a growing zone *ED*.

The present paper aims to give an account of the nuclear changes, both degenerative and regenerative, involved in the formation of the regenerated spinal cord.

II. Material and Methods.

Serial sections were made of tadpole tails killed after various regeneration periods. This enables one to follow the process from stage to stage. But to get uniform results from this method and eliminate individual variations, one must take tadpoles as nearly alike as possible at the start, operate on all at the same time, keep them under uniform laboratory conditions and make sections of several individuals at each stage.

On October 12, 1913, seventy tadpoles of *Rana clamitans*, varying in length from 30 to 60 mm., were brought into the laboratory. Two days later they were put into individual finger bowls, and forty-four medium sized individuals (32–40 mm. in length), chosen to constitute the main series, were grouped by twos or threes. Those of each group were as nearly alike as possible and each group was treated as a unit in the time of operation, killing, etc. The finger bowls were placed side by side on a table some distance from the windows so that uniform conditions of temperature, light, etc., were insured. None of the tadpoles was fed during the course of the experiment, and none died from the effects of laboratory conditions.

On October 15, the first operations were performed. Each tadpole was transferred from the finger bowl to a paraffin block and approximately one fourth of the tail was removed, with a sharp scalpel, at right angles to the plane of the tail. The animal was returned to the finger bowl and the removed part put into Gilson's killing fluid. At the end of the period of regeneration, the animals were again taken out onto the block and the

regenerated tail plus a second fourth of the normal tail was removed and put immediately into Gilson's killing fluid. The times of killing were as follows: normal, immediately after the operation, 1, 3, 5½, 9½, and 14 hours, and 1, 2, 3, 4, 6, 8, 9, 10, 12, 14 and 16 days after the operation. Usual methods of technique were followed. Delafield's hæmatoxylin and acid fuchsin stain the nuclei blue and the cytoplasm pink, but do not distinctly bring out cell boundaries. For the most part sections were made in the sagittal plane.

III. Observations.

The study was confined to the histology of regeneration in the spinal cord, since a preliminary examination showed that this organ of all those in the tail was best adapted for a study of the present problem. Fig. 2 shows by a sagittal section the spinal

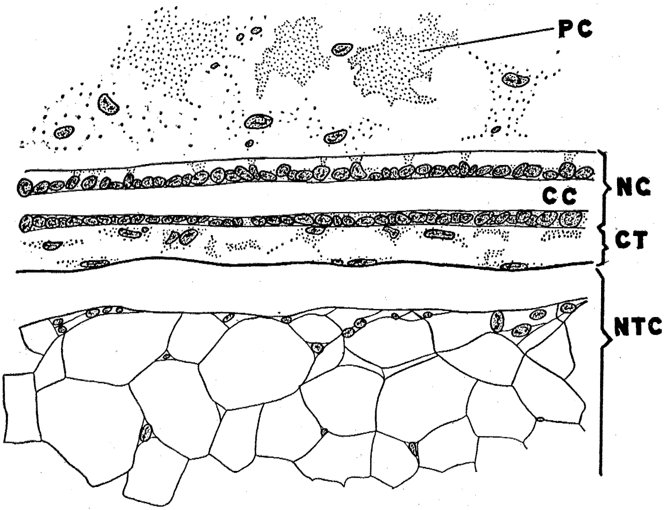

FIG. 2. Sagittal section through a part of the normal tail, showing the spinal cord and its relation to the surrounding tissues. *nc*, spinal cord; *cc*, central canal; *ntc*, notocord; *ct*, connective tissue; *pc*, pigment cell. (330 diameters.)

cord, and its relation to the surrounding tissues. Fig. 3 shows a transverse section of the spinal cord alone. It is a hollow tube which distally is formed of a single layer of cells. The nuclei are very near the inner border of the cells so that there is a wide outer zone of cytoplasm but practically no inner cytoplasmic

zone. At this stage in the development of the tadpole, the cells near the distal end of the spinal cord show little differentiation.

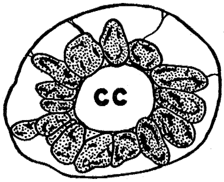

FIG. 3. Transverse section through the normal spinal cord, showing the nuclei and the outer cytoplasmic zone. *cc*, central canal. (890 diameters.)

1. *Degenerative Changes after an Operation.*

When a tadpole's tail is removed the old notocord extends out beyond the other tissues, and the connective tissue between the notocord and spinal cord is usually broken so that the spinal cord bends dorsally as in Figs. 7 and 8. A transverse cut through the tail leaves the various organs at the cut surface in contact with the surrounding medium, the water in which the tadpole lives. Sections of tadpoles killed immediately after the operation show the direct effect of the cutting (Figs. 4 and 5). Many

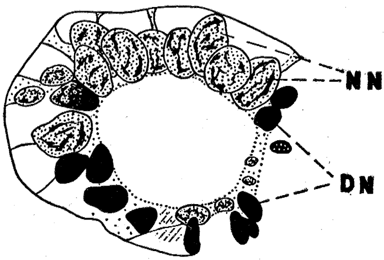

FIG. 4. Transverse section through the end of the spinal cord immediately after the operation, showing deeply-staining nuclei. *cc*, central canal; *nn*, normal nuclei; *dn*, deeply-staining nuclei. (920 diameters.)

nuclei and cells are broken and irregular in appearance and may be loosened or torn apart from each other. The injured nuclei at the cut edge and extending forward with decreasing frequency, are homogeneous in appearance and take a deep haematoxylin stain. Undoubtedly some of the nuclei are cut, and this accounts for the irregularity in shape of a good many. But a good many others, also staining deeply, are rounded and smaller than normal nuclei. These may be either normal nuclei which under the

stimulus of the operation are contracted or compressed, or cut nuclei which have rounded off. These deeply-staining nuclei, whether rounded or irregular in shape, are smaller than normal

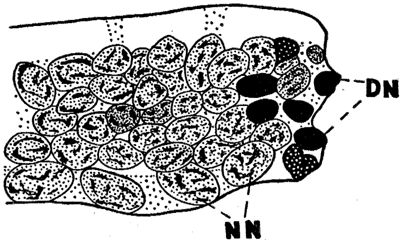

FIG. 5. Sagittal section through the side of the spinal cord immediately after the operation, showing the deeply-staining nuclei at the cut end. *dn*, deeply-staining nuclei. (920 diameters.)

nuclei, so it may be that the chromatin, which stains deeply, is condensed on account of the loss of achromatic material.

The same assumption is borne out by the somewhat different appearance of nuclei in the tadpoles killed one hour after the operation (Fig. 6). Some are rounded as before; others are angular or slightly hour-glass shaped, with rather dense cytoplasm extending out from the corners. If parts of the nuclear

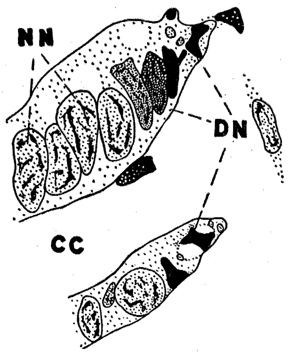

FIG. 6. Sagittal section through the spinal cord one hour after the operation. This shows the "contracting" nuclei. *cc*, central canal; *dn*, deeply-staining nuclei; *nn*, normal nuclei. (920 diameters.)

membrane were held by the cytoplasm while the nucleus as a whole decreases in volume either by contraction or loss of achromatin, the nuclei might present such an appearance. Moreover there are gradations from hour-glass-shaped to normal nuclei

and corresponding gradations in size and depth of stain. In cases of this sort there are often vacuoles or cytoplasm between the nuclei as if the latter had shrunken, whereas in the normal cord, the nuclei are so close together that no cytoplasm can be seen between them. These facts indicate that normal nuclei become deeply staining nuclei by contraction or by loss of achromatic material.

This "contraction" of nuclei seems to be caused by contact with the water or killing fluid, or the succession of the two, as well as by direct injury from the scalpel, for other nuclei which are in contact with the exterior only through the central canal show this phenomenon. In some cases, the end of a nucleus nearest the central canal is deeply stained and contracted while the other part is normal (Fig. 4). The question immediately arises, why does not the water or other external factor enter the open neural tube and cause the contraction of the inner parts of practically all nuclei in the spinal cord? It is probably because of the presence in the tube of some substance which prevents the ready admission of external fluids, though capillarity would have a similar effect. Since the sections show very little structure within the central canal, this content must be liquid or semi-liquid. However, in a number of sections there is a rather long narrow band of cytoplasmic material which may be the more solid part of a semi-liquid substance coagulated by the killing reagent. There are other evidences of the presence of such a liquid. The sections from two of the tadpoles killed one hour after the operation show a coagulation of the outer surface of the blood plasma covering the wound, but over the spinal cord this coagulating process is delayed. The most plausible explanation seems to be that some cerebro-spinal fluid (compared by Barfurth to the cerebrospinal fluid of mammals) exerts an outward pressure which breaks through any slight hardening of the plasma at this point. Perhaps transference of the animal to a medium of different density, the killing fluid, aids the outburst. Sections of another tadpole killed at one hour show the presence of this coagulated plasma over the end of the spinal cord as well as over other parts of the tail.

The outward pressure of a fluid would tend to push out into

the blood plasma any free elements such as the injured and degenerating nuclei with very little cytoplasm and hence little connection with other cells; and when this fluid breaks through, some of these nuclei may break off and float away. At one hour after the operation, broken and small rounded nuclei are seen in

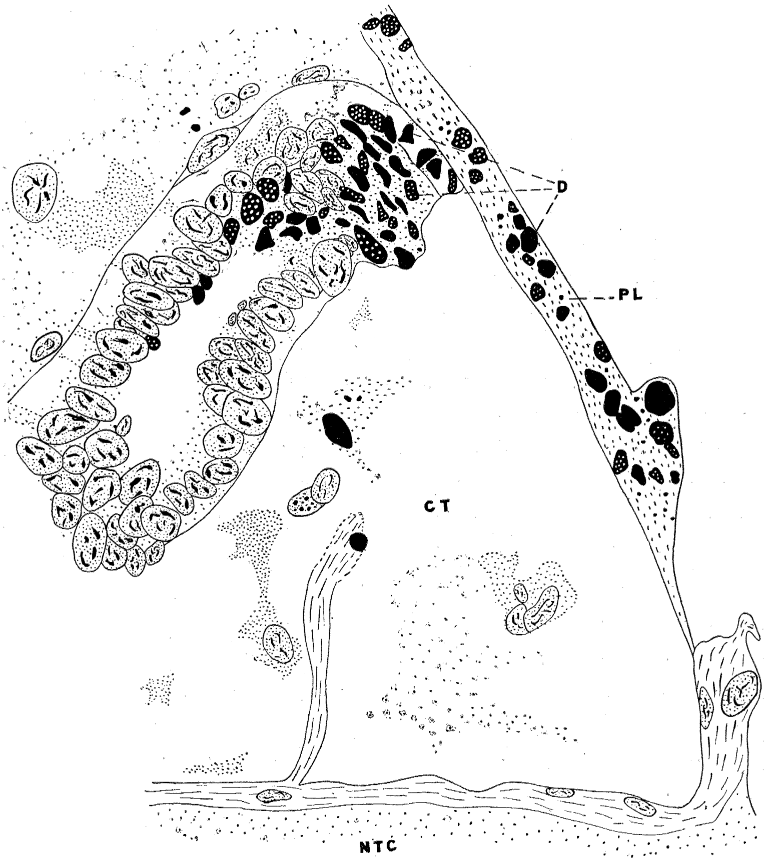

FIG. 7. Sagittal section through the spinal cord and the surrounding region one hour after the operation, showing irregularly shaped, deeply-staining nuclei in the end of the spinal cord and in the coagulated plasma layer. *dn*, deeply-staining nuclei; *pl*, plasma layer; *ct*, connective tissue; *ntc*, notocord. (1,100 diameters.)

the end of the spinal cord and extending out into the hardened layer of the plasma, giving evidence of some force acting outward at this time (Fig. 7). Other evidences will be mentioned in describing the stages at which they appear.

Three hours after the operation there are fewer of the angular nuclei than at one hour and more of the round deeply-staining nuclei. The latter vary from the size of similar ones in the earlier stages down to fragments. Moreover some of the larger of these seem to be in the process of fragmentation, that is, appearances indicating stages in direct division are seen. The gradation in size and depth of stain at one hour from normal nuclei nearly to rounded ones, and the gradation down to fragments at three hours, as well as the appearances of fragmentation, make it fairly clear that normal nuclei just in front of the cut edge may contract, become rounded, and fragment. This must be a degenerative process. Even finer intermediate steps are seen in preparations of later stages.

Sections of one individual at this period appear very much like those immediately after the operation. The deeply-stained nuclei are similar, and the spinal cord is not covered either by epidermis or plasma, so that a recent outbreak of the cerebrospinal fluid must have taken place. In this case a second contact with the exterior has again started the degenerative process.

At five and a half hours the spinal cord is entirely covered by the thickened plasma layer, in which is a group of fragmenting globular nuclei. In one preparation at this time, the epidermis has closed-in over the entire wound, and there is a series of stages in the degeneration of nuclei. Some are only slightly smaller and darker than normal nuclei; others have the angular appearance characteristic of nuclei one hour after the operation, while still others are round and fragmenting. At this stage there is another evidence of the presence of a cerebrospinal fluid. The plasma covering the end of the spinal cord is pushed outward, making a knob-like extension of the central canal similar to that shown in Fig. 8. This did not appear in earlier stages either because not enough cerebrospinal fluid was present, or because the plasma layer had not coagulated sufficiently to resist the outward pressure of this fluid.

Of the two preparations of tadpoles killed after a nine and a half hour interval, one shows the epidermis and plasma covering all the wound except the neural tube; the other shows this part also covered. In the former, the sides of the neural tube are

separated as if by a recent outburst of cerebrospinal fluid, and deeply-staining rounded and fragmenting nuclei are seen. In the second preparation, the deeply-staining nuclei are all small

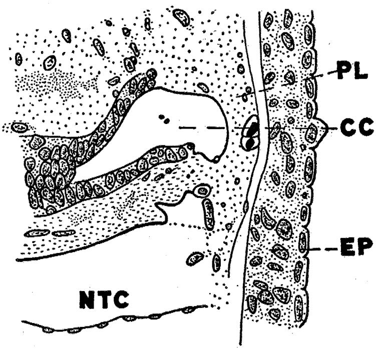

FIG. 8. Sagittal section through the end of the spinal cord fourteen hours after the operation. This shows the epidermal layer, the plasma layer, and the knob-like extension of the central canal, caused by the outward pressure of the cerebrospinal fluid. *ep*, epidermis; *cc*, central canal; *pl*, plasma layer; *ntc*, notocord. (330 diameters.)

and fragmentary. In other words no more nuclei seem to be starting to degenerate.

At fourteen hours, plasma and epidermis cover the spinal cord though the plasma is pushed outward by the cerebrospinal fluid (Fig. 8). There are nuclear fragments in the cord and degenerating nuclei in the plasma. Another preparation of the same period shows the nerve cord still open to the exterior, as well as the nuclear appearance of an earlier stage.

At twenty-four hours, only a few of the nuclei are slightly smaller and darker than the normal. At this time there appear near the end of the spinal cord, granular leucocytes containing pigment granules and fragments which closely resemble the fragments of degenerating nuclei. It may be that the leucocytes appear at this time and dispose of nuclear fragments. After one day, the degenerating nuclei are too rare to be significant.

The degenerative process which the foregoing facts seem to show, may be indicated diagrammatically as follows:

Cells directly cut → broken nuclei → rounded nuclei → fragments → disposed of by outbreak of cerebro-spinal fluid, or by leucocytes.

Cells just in front of those cut → angular nuclei → rounded nuclei → fragments → disposed of by leucocytes.

2. *Enlargement of Nuclei.*

A few preparations of the spinal cord soon after the operation show plainly that the nuclei near the end, but just in front of the deeply-staining nuclei, are larger than those of the normal cord. The long axes of nuclei close to the edge were measured and compared to nuclei of the same preparation which are some distance forward in the old tissue (Table I.). Immediately after the operation and in the very early regeneration stages, the nuclei near the end are larger, but the difference decreases until after nine and a half hours it is hardly significant. This enlargement might be preparatory to normal division or it might be a swelling which is a degenerative change preliminary to fragmentation. Since this size difference is greatest at the very beginning and decreases during the first day until it is no longer significant, and since mitotic divisions are not seen in numbers until the third day, the enlargement is probably an early stage in nuclear degeneration.

TABLE I.

Time of Regeneration.	Nuclear Length Close to Edge.	Nuclear Length in Front of Edge.	Difference in Length.
Normal	7.9	7.5	.4
Immediately	10.5	7.8	2.7
1 hour	11.3	9.1	2.2
3 hours	8.6	7.3	1.3
5.5 hours	12.8	10.5	2.3
9.5 hours	8.1	8.0	.1
14 hours	7.6	8.3	−.7
1 day	8.2	6.6	1.6
2 days	8.1	8.0	.1
3 days	8.2	8.9	−.7
4 days	8.4	8.2	.2
6 days	10.7	10.4	.3

Explanation.—Each measurement recorded here is the average of the measurements of 9 or 10 nuclei. These were recorded in terms of the spaces of the ocular micrometer, but since one space was equal to approximately one micron (.955), the measurements were not transposed.

3. *Temporary Partial Closing of the Spinal Cord.*

After the degenerative process is complete and the deeply-staining nuclei have disappeared, the end of the nerve cord starts to close over. By the first day, the nuclei in the end of the cord

have begun to pull apart, stretching out the connecting cytoplasm (Fig. 11). In general they extend toward the opposite

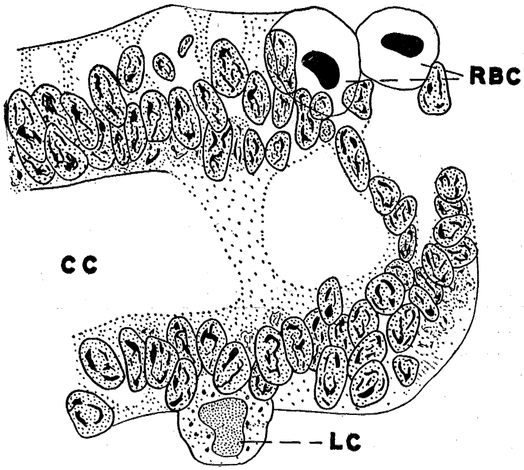

FIG. 9. Sagittal section close to the edge of the central canal, showing a row of cells, not quite at the end, extending across the central canal. Other sections of the series show that the end of the cord is still open. *cc*, central canal; *rbc*, red blood corpuscles; *lc*, leucocyte. (920 diameters.)

wall of the central canal, thus narrowing the opening at the end. Some sections show pseudopod-like cytoplasmic extensions of the cells into the central canal as if closing were to be produced

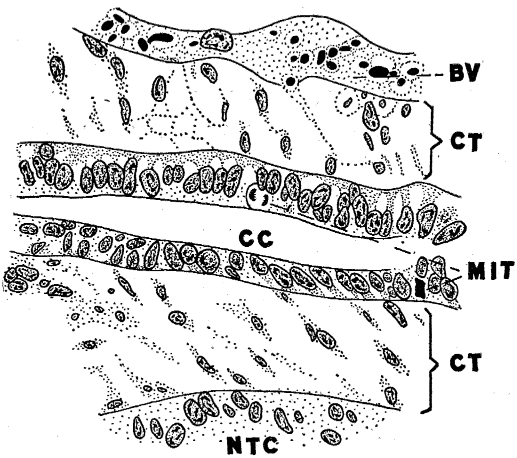

FIG. 10. Sagittal section through the new spinal cord six days after the operation. *bv*, blood vessel; *mit*, mitotic figures; *ntc*, notocord; *ct*, connective tissue. (330 diameters.)

by amoeboid movement of the cells. Figure 9 shows a section through one side of the cord, in which one layer of cells, not quite at the end, is extending down into the central canal. Up to about six days phenomena such as these may be seen, but sections from six to sixteen days show that the closing is not completed within that period. By sixteen days the new tail is

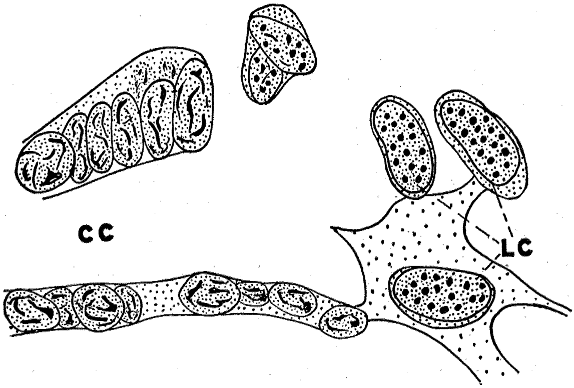

FIG. 11. Sagittal section through the spinal cord one day after the operation showing the granular leucocytes at the end of the cord, and the pulling apart of nuclei in the lower part of the cord. *cc*, central canal; *lc*, leucocytes. (920 diameters.)

almost as long as it will become (Durbin, 1909), and the spinal cord reaches back close to the epidermis at the posterior end. Still these later preparations show the sides of the neural tube gaping open, and red blood corpuscles extending forward into the central canal of the new cord, as if the pressure of the cerebrospinal fluid is not sufficient to keep them out.

4. *Cell Division.*

In an organ such as the spinal cord in which the nuclei lie close together, it is difficult to determine an amitotic division. In order to be sure that amitotic divisions do occur, one must find continuous stages in nuclear and cellular constriction without the formation of chromosomes. Because of the massing of nuclei, this cannot readily be determined in the normal spinal cord, though the slides were examined with this point in mind. The present study gives no evidence that normal nuclei divide amitotically, but stages in direct division can be seen in the

deeply-staining nuclei at the cut edge. Is this amitosis or fragmentation? Do the daughter nuclei form normal nuclei, or do they divide several times and degenerate? There is no definite evidence that nuclei which divide directly ever become normal again. But at successive stages the deeply-staining nuclei become smaller and smaller down to fragments, so that the direct division is probably a fragmentation as a part of the degeneration of injured nuclei.

Mitotic divisions can easily be distinguished by the formation of chromosomes. All the preparations were examined and the distance of each mitotic division from the cut edge was measured. The results are shown in Table II. In the sections of the normal tail the number of divisions is the smallest, but since up to three days the mitoses are scattered and the number of individuals small, there is no reason for considering these mitoses anything but normal. During the period of degenerating nuclei, there are almost no mitotic divisions close to the edge. On the third day, the nuclei just in front of the cut edge are proliferating rapidly; at four days there are a few divisions past the cut; at six days there are almost as many divisions in the new spinal cord as in the old; at eight and nine days most of the divisions are in the new cord; at fourteen days there are scattered mitoses only, both in the old and new cord, and at sixteen days most of the dividing cells are in the very end of the new cord. If later divisions follow this general trend, it seems likely that the rest of the spinal cord will be formed by a growing zone at the tip, and until the new cord is complete the number of mitoses near the tip would probably decrease gradually.

Fig. 12 gives the average number of mitoses in the spinal cord at each stage, and therefore represents the rate of growth at these times. On the second day there is a considerable mass of tissue over the whole wound, though only degenerative changes have been taking place in the nerve cord. Beginning about this time, the nuclei in the end of the cord loosen and draw apart somewhat, stretching out the cytoplasm between them (Fig. 11). This is apparently the first extension in length of the spinal cord. At three days active proliferation of cells has begun but the pulling apart or stretching toward the cut edge continues. Fig.

TABLE II.

Regeneration Time.	No. of Individuals.	Mitoses in Old Cord.	Mitoses in New Cord.	2.8 mm.	2.1 mm.	1.4 mm.	.7 mm. ← Old Tissue.	Position of Cut. New Tissue. →	.7 mm.	1.4 mm.
Normal..	3	$\frac{2}{3}$	0		$\frac{1}{3}$	$\frac{1}{3}$				
Immed...	3	$2\frac{2}{3}$	0	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$ $\frac{1}{3}$ $\frac{2}{3}$	$\frac{1}{3}$ $\frac{1}{3}$			
1 hr.	3	6	0	$\frac{2}{3}$ $\frac{2}{3}$	$\frac{2}{3}$ 1 $\frac{1}{3}$	$\frac{1}{3}$ $\frac{1}{3}$ $\frac{2}{3}$	$\frac{1}{3}$ $\frac{1}{3}$ $\frac{2}{3}$			
3 hrs.....	2	$1\frac{1}{2}$	0	1	$\frac{1}{2}$					
$5\frac{1}{2}$ hrs....	2	$2\frac{1}{2}$	0	1 $\frac{1}{2}$		$\frac{1}{2}$	$\frac{1}{2}$			
$9\frac{1}{2}$ hrs....	2	$1\frac{1}{2}$	0		$\frac{1}{2}$ $\frac{1}{2}$	$\frac{1}{2}$				
14 hrs....	2	$3\frac{1}{2}$	0	$\frac{1}{2}$	$\frac{1}{2}$ $\frac{1}{2}$	1 $\frac{1}{2}$	$\frac{1}{2}$			
1 day....	3	$1\frac{1}{3}$	0		$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$ $\frac{1}{3}$			
2 days...	4	$1\frac{1}{3}$	0		$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$ $\frac{1}{3}$			
3 days...	1	19	0				6 4 8 1			
4 days...	2	15	$2\frac{1}{2}$	$\frac{1}{2}$ $\frac{1}{2}$ 1	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$ 3 $2\frac{1}{2}$ 6	1 $1\frac{1}{2}$		
6 days...	2	$19\frac{1}{2}$	19	$\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$		1 1 $\frac{1}{2}$ 2	$2\frac{1}{2}$ 2 $2\frac{1}{2}$ 4 $2\frac{1}{2}$	4 $3\frac{1}{2}$ $2\frac{1}{2}$ $6\frac{1}{2}$ $2\frac{1}{2}$		
8 days...	1	2	40				1 1	3 12 6 5 4	1 3 6	
9 days...	1	16	42			1 1 1 3	3 1 6	2 7 4 5	3 3 4	9 5
14 days..	2	6	6		$\frac{1}{2}$	$\frac{1}{2}$ $\frac{1}{2}$ $1\frac{1}{2}$	$1\frac{1}{2}$ 1 $\frac{1}{2}$	1 1 $\frac{1}{2}$ 1	$\frac{1}{2}$ 1 1	
16 days..	2	$\frac{1}{2}$	$7\frac{1}{2}$			$\frac{1}{2}$		1 $\frac{1}{2}$		3 3

This table represents the position and number of mitotic divisions taking place at different times. In most cases the number given is the average obtained from 2 to 4 individuals, as indicated in the table.

10 (six days' regeneration) shows the cells in one part of the cord stretched out to such an extent that vacuoles are left between the

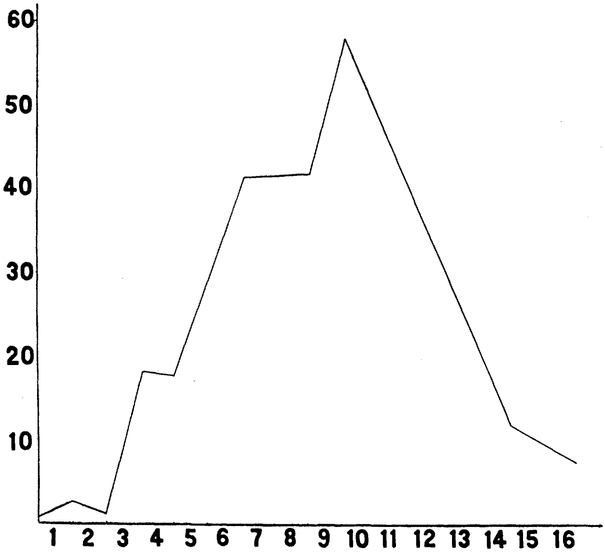

FIG. 12. Curve giving the number of mitotic divisions in that part of the spinal cord within 3 mm. of the edge. Beyond 3 mm. the mitoses are scattered. An abscissa represents the period of regeneration and the corresponding ordinate gives the average number of mitoses found in the individuals killed at the end of that period.

cells. It is during the period from four to sixteen days that most of the increase in length takes place, by active proliferation and migration of cells.

IV. Discussion.

1. *Amitosis and Fragmentation.*

Fraisse in describing the regulative process at about two days after the operation, says; "Bereits früher machte ich darauf aufmerksam, dass am Wundrande eine starke Auswanderung von Leukocyten stattfindet, und dass diese es sind, welchen vor allen Dingen die Bildung des homogenen, lymphartigen Saumes, welcher zuerst die wunde bedeckt, zuzuschreiben ist. Das Rückenmark geht nun an meinen Schnitten bis dichte an diesen homogenen Saum heran, und die Elemente, welche es zusammensetzen, lassen sich immerhin noch nach 24 Stunden auch an

diesem Saum von einander trennen, dann aber tritt eine bedeutende Wucherung von Kernen auf, und zwar scheint dieselbe auszugehen von den sogenannten Körnern,[1] deren Inhalt völlig homogen und stark lichtbrechend erscheint. Durch Picrocarmin werden diese Elemente ebenfalls stark tingirt, und nun sieht man an diesen nahezu gleich grossen Körnern Kerntheilungen, ohne dass jemals eine Spur von karyokinetischen Figuren constatirt werden konnte, in der Weise auftreten, das der Kern oder die Körner sich in der bekannten Weise schuhsohlenförmig einschnüren, und dass dann aus beiden Hälften Elemente gleicher Art hervorgehen. Nicht nur eine einmalige Einschnürung glaube ich beobachten zu können, sondern auch eine mehrfache, so dass der Kern sich bei diesem Process nicht nur in zwei, sondern auch in mehrere Stücke theilen kann."

Fraisse discusses further the evidence that the nuclei from the end of the spinal cord, which are found in the lymph-like border, divide amitotically. This agrees with the present observations. But he is satisfied to show that direct division does take place. So far as my preparations show, there are few evidences that the nuclei which divide amitotically afterward become normal nuclei. In some of the preparations of stages at which the deeply-staining nuclei have almost disappeared, there are a few nuclei which stain only slightly darker than the normal ones, and at this time there are no stages between these and the fragments. These few slightly darkened nuclei may, then, be forming normal nuclei again. All other evidence points towards the conclusion that at successive stages, these deeply-staining nuclei become smaller and smaller as if fragmentation or repeated direct division, is taking place. The conclusion from these facts is that nuclei which have only started to degenerate may perhaps return to the normal condition, but that nuclei that have gone so far as to divide amitotically are destined to fragment.

2. *The Appearance of Leucocytes.*

Barfurth (1891), working on the regenerating spinal cord of the frog larva at forty-six hours and at three days, makes the

[1] Körnern-nuclei of the gray substance, which are not present in the distal region of the spinal cord.

following statement: "Die unterste Theil des regenerirten Medullarrohres beherbergt in seinem Innern und zwischen seinen Epithelzellen zahlreiche fettig degenerirende Leukocyten; viele kleine und grosse Fetttropfen, die man hier überall findet, führe ich ihrem Ursprunge nach auf solche zerfallene Wanderzellen zurück. Ausserdem finden sich hier auch viele Pigmentkörnchen, die wohl bei der regressiven Metamorphose der zerfallenden Leukocyten entstehen (Pigmententartung)."

Barfurth figures the spinal cord of a larva of *Triton cristatus* after the sixth day of regeneration, in which these leucocytes and fat drops are shown. His figure is very similar to Fig. 11, which shows a section of a tadpole killed twenty-four hours after the operation. Both Fraisse and Barfurth mention particularly the presence of leucocytes in the early regeneration stages, but in the present study, leucocytes were not found in large numbers. Up to the end of the first day, none at all were seen close to the spinal cord. The earliest stage mentioned by Barfurth is that after a forty-six hour regeneration period, and this probably accounts for the different interpretation he gives of the origin of the "Fetttropfen" or fragments. If these fragments are followed back into earlier stages in my sections, they become larger and larger and are seen to be identical with the degenerating nuclei. To be sure, the leucocytes when they first appear in the spinal cord region contain what might be called fat drops, but is it not more reasonable to suppose that the leucocytes which are present at this time dispose of the fragments of injured spinal cord nuclei?

3. *Temporary Closing of the Spinal Cord.*

Barfurth describes the closing of the spinal cord at three days by means of cytoplasmic extensions of the cells, such as were seen in the preparations used in the present study. "Der sich wieder ansammelnde Liquor cerebrospinalis drückt nun auf die neugebildeten, noch wenig resistenten untern und seitlichen Theile des Rohres, und treibt sie kolbenartig auseinander. Die Zellen passen sich einstweilen durch ihre Lagerung diesem Druck an und behalten später diese Lage noch eine Zeit lang bei." Barfurth mentions this as a temporary closure of the spinal cord, so

his later preparations evidently show the cord again open. The regenerated spinal cord at sixteen days has almost reached its maximum length, but it is not yet closed. Whether or not the completely regenerated spinal cord is open at the end or closed as in the normal tail cannot be answered by the present study.

4. *Rate of Division. Amitosis versus Mitosis.*

Durbin (1909), in analyzing the rate of increase in length throughout the regenerative process in the tail of *Rana clamitans*, distinguishes four periods. "The operation was followed by an interval of low rate, succeeded by one of rapidly increasing rate, then by one of rapidly decreasing rate and finally an interval in which the rate gradually approaches zero. The first low period is explained by a combination of two factors, (*a*) the shock of the injury, and (*b*) the formation of a cap of embryonic cells which is to serve as a basis for the more active regeneration. The second or period of rapidly increasing growth is the one in which practically all the cells in the new part are undifferentiated and rapidly dividing. The third and fourth periods are explained by the appearance of differentiation, which lessens the number of dividing cells."

Fig. 12, based on the number of mitotic divisions in the spinal cord, shows these same periods. The initial period of low rate covers the first two days; that of rapidly increasing rate includes the third to ninth days; the period of rapidly decreasing rate extends from the tenth to sixteenth days, and the period of gradually decreasing rate, though not covered in the present work, would undoubtedly extend on from about sixteen days. In the light of this histological study, a somewhat different interpretation might be given to the initial period. It is during these first two days that degeneration of the injured cells is taking place. Though at this time a cap of undifferentiated cells is being formed over the wound, the spinal cord does not participate in the formation of this cap, nor is any such cap formed at the end of the spinal cord. Since the spinal cord cells in this part of the tail are so slightly differentiated, the new cord is formed from the old without the separation of a group of special embryonic cells.

The similarity of the rate curves based on a counting of the mitotic divisions with that based on the amount of tissue formed at each period, seems to be significant. It shows that the rate of tissue formation is closely correlated with the number of mitotic divisions. Considering amitosis, this may be interpreted in one of two ways—(1) either the number of amitotic divisions is similarly correlated with the rate of growth so that the total number of divisions both mitotic and amitotic, gives the same form of curve as the mitotic divisions alone, or else (2) amitotic divisions are not numerous enough to be significant. The former explanation is improbable. The nuclear conditions producing mitotic division are probably different from those producing amitotic division. Different cells in the same region may divide by different methods, but it is very improbable that the conditions producing one form of division would increase and decrease in influence at the same rate and the same times as those producing the other form. Moreover, in the present study, no examples of direct division were seen except in the degenerating, fragmenting nuclei. This similarity of the rate curve of mitotic divisions to the rate curve of growth is evidence, other than the negative observational evidence, supporting the view that amitotic division is not important in the formation of this organ by regeneration.

V. Summary.

1. The regenerating spinal cord of the frog tadpole has been studied histologically in order to learn the mechanism, or the stages in the process, by which the new cord is formed from the old.

2. During the first day after the operation, injured nuclei in the end of the spinal cord degenerate. There is first a decrease in size, by contraction or loss of achromatin, and then a fragmentation of these degenerating nuclei. The fragments may be carried away either by the outbreaking of a cerebrospinal fluid or by leucocytes which appear at this time. These fragments are parts of disintegrated spinal cord nuclei and not of leucocytes.

3. From the second to the sixth days there is a temporary partial closing of the neural tube, probably by migration of the cells near the end.

4. The new cord is formed by the cells of the old cord near the cut edge, by mitotic division and migration.

5. The number of mitotic divisions at different periods is proportional to the rate of regeneration at those periods as determined by Durbin. Amitotic division, if it occurs, is not important in the formation of the regenerated organ.

6. There is no observational evidence from this study that amitotic division does occur in normal regenerating spinal cord cells.

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